

Claims

What is claimed is:

- 5 1. A method of identifying compounds that affect p53 stability, the method comprising the steps of:
 - providing a cell line transfected with a construct expressing p53-fused to a reporter protein and a control reporter protein;
 - contacting a test compound with cells from the cell line;
 - 10 contacting cells with a DNA damaging agent; and
 - comparing levels of the p53 fusion protein and the control reporter protein.
- 15 2. A method of identifying compounds that affect p53 stability, the method comprising the steps of:
 - providing a cell line transfected with a construct expressing p53-fused to a reporter protein and a control reporter protein, and transfected to express E6;
 - contacting a test compound with cells from the cell line;
 - contacting cells with a DNA damaging agent; and
 - 20 comparing levels of the p53 fusion protein and the control reporter protein.
3. The method of claim 1 wherein the cell line is derived from a cell line known to contain active p53 turnover pathways.
4. The method of claim 1 wherein the cell line is derived from a cell line that has a
25 relatively low steady state level of p53 protein and shows a significant accumulation of p53 protein following treatment with a DNA damaging agent.
5. The method of claim 1 wherein the DNA damaging agent is a chemotherapeutic agent.

6. The method of claim 1 wherein the DNA damaging agent is adriamycin.
7. The method of claim 1 wherein the cell line is an animal cell line.
- 5 8. The method of claim 1 wherein the cell line is a mammalian cell line.
9. The method of claim 1 wherein the cell line is a human cell line.
10. The method of claim 1 wherein the cell line is an RKO cell line.
- 10 11. The method of claim 1 wherein the cell line is an MCF7 cell line.
12. The method of claim 1 wherein the p53 fusion protein is detectable by an antibody.
- 15 13. The method of claim 1 wherein the control reporter protein is detectable by an antibody.
14. The method of claim 1 wherein the p53 fusion protein is detectable by a change in absorbance, change in fluorescence, or radio-immuno assay.
- 20 15. The method of claim 1 wherein the control reporter protein is detectable by a change in absorbance, change in fluorescence, or a radio-immuno assay
16. The method of claim 1 wherein the p53 fusion protein and the control reporter protein are translated from a single mRNA transcript.
- 25 17. The method of claim 16 wherein an internal ribosome entry site is inserted between a gene encoding the p53 fusion protein and a gene encoding the control reporter protein.

18. The method of claim 1 wherein a gene encoding the p53 fusion protein and a gene encoding the control reporter protein are controlled by a same promoter sequence and regulatory sequences.

5 19. The method of claim 1 wherein the reporter protein or the control reporter protein is selected from the group consisting of firefly luciferase, secreted alkaline phosphatase, enhanced green fluorescent protein, horseradish peroxidase, beta-galactosidase, and renilla luciferase.

10 20. The method of claim 1 wherein the reporter protein or the control reporter protein is selected from the group consisting of firefly luciferase and renilla luciferase.

21. The method of claim 1 wherein the p53 fusion protein and the control reporter protein are transcribed at substantially the same level.

15 22. The method of claim 1 wherein the construct comprises a gene encoding a p53/FL (firefly luciferase) fusion protein, and a gene encoding RL (renilla luciferase) protein.

20 23. The method of claim 22 wherein the construct further comprises a CMV promoter controlling the expression of the gene encoding the p53/FL fusion protein and the gene encoding the RL protein, and an internal ribosome entry site.

24. A kit useful in identifying compounds that affect p53 stability comprising:
a cell line with a construct expressing p52-fused to a reporter protein and a control reporter protein.

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25. A method of identifying compounds that affect stability of a protein of interest, the method comprising steps of:

providing a cell line transfected with a construct expressing a first reporter protein fused to the protein of interest, and a second control reporter protein;

contacting a test compound with cells from the cell line;
contacting the cells with a DNA damaging agent; and
comparing levels of the first reporter protein and the second reporter protein.

- 5 26. A method of identifying compounds that affect stability of a protein of interest, the
method comprising steps of:
- providing a cell line transfected with a construct expressing a first reporter protein fused
to the protein of interest, and a second control reporter protein;
- contacting a test compound with cells from the cell line;
- 10 contacting cells with a DNA damaging agent; and
- comparing levels of the first reporter protein and the second reporter protein.